



US010619218B2

(12) **United States Patent**
McCord et al.

(10) **Patent No.:** **US 10,619,218 B2**
(45) **Date of Patent:** ***Apr. 14, 2020**

(54) **MATERIALS AND METHODS FOR
DETECTING SOURCE BODY FLUIDS**

(71) Applicants: **Bruce McCord**, Miami, FL (US);
Joana Antunes, Miami, FL (US)

(72) Inventors: **Bruce McCord**, Miami, FL (US);
Joana Antunes, Miami, FL (US)

(73) Assignee: **THE FLORIDA INTERNATIONAL
UNIVERSITY BOARD OF
TRUSTEES**, Miami, FL (US)

(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 16 days.

This patent is subject to a terminal dis-
claimer.

(21) Appl. No.: **16/054,210**

(22) Filed: **Aug. 3, 2018**

(65) **Prior Publication Data**

US 2019/0284643 A1 Sep. 19, 2019

Related U.S. Application Data

(62) Division of application No. 15/921,079, filed on Mar.
14, 2018, now Pat. No. 10,053,740.

(51) **Int. Cl.**

C07H 21/04 (2006.01)

C12Q 1/68 (2018.01)

C12Q 1/6888 (2018.01)

C12Q 1/6881 (2018.01)

(52) **U.S. Cl.**

CPC **C12Q 1/6888** (2013.01); **C12Q 1/6881**
(2013.01); **C12Q 2600/154** (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

(56) **References Cited**

PUBLICATIONS

Yoshino et al. (J. of Alzheimer's Disease, vol. 58, pp. 687-694,
2017) (Year: 2017).*

An, J. H. et al., "Body fluid identification in forensics." BMB
Reports, Sep. 2012, 45 (10): 545-553.

Antunes, J. et al., "Forensic discrimination of vaginal epithelia by
DNA methylation analysis through pyrosequencing." Electropho-
resis, 2016, 37: 2751-2758.

Antunes, J. et al., "High-resolution melt analysis of DNA methylation
to discriminate semen in biological stains." Analytical Biochemis-
try, 2016, 494: 40-45.

Antunes, J. et al., "Tissue-specific DNA methylation patterns in
forensic samples detected by Pyrosequencing®." Pyrosequencing,
Department of Chemistry and Biochemistry, Florida International
University, School of Criminal Justice, the University of Southern
Mississippi, 2014, 1-21.

Du, P. et al., "Comparison of Beta-value and M-value methods for
quantifying methylation levels by microarray analysis." BMC Bio-
informatics, 2010, 11 (587): 1-9.

Eads, C. A., Laird, P. W., "Combined Bisulfite Restriction Analysis
(COBRA)." Methods in Molecular Biology, 2002, 200: 71-85.

Ehrich, M. et al., "Quantitative high-throughput analysis of DNA
methylation patterns by base-specific cleavage and mass spectrom-
etry." PNAS, Nov. 2005, 102 (44): 15785-15790.

Fraga, M. F. et al., "Epigenetic differences arise during the lifetime
of monozygotic twins." PNAS, Jul. 2005, 102 (30): 10604-10609.

Grškovć, B. et al., "DNA methylation: the future of crime scene
investigation?" Mol. Biol. Rep., 2013, 40: 4349-4306.

Haas, C. et al., "mRNA profiling for the identification of blood—
Results of a collaborative EDNAP exercise." Forensic Science
International: Genetics, 2011, 5: 21-26.

Harbison, S. A., Fleming, R. I., "Forensic body fluid identification:
state of the art." Research and Reports in Forensic Medical Science,
2016, 6: 11-23.

Juusola, J., Ballantyne, J., "mRNA Profiling for Body Fluid Iden-
tification by Multiplex Quantitative RT-PCR." J. Forensic. Sci.,
Nov. 2007, 52 (6): 1252-1262.

Li, L., Dahiya, R., "MethPrimer: designing primers for methylation
PCRs." Bioinformatics, May 2002, 18 (11): 1427-1431.

Park, J. et al., "Identification of body fluid-specific DNA methylation
markers for use in forensic science." Forensic Science International:
Genetics, 2014, 13: 147-153.

Paul, C. L., Clark, S. J., "Cytosine Methylation: Quantitation by
Automated Genomic Sequencing and GENESCAN Analysis." Bio-
Techniques, Jul. 1996, 21 (1): 126-133.

Tost, J., Gut, I. G., "Analysis of Gene-Specific DNA Methylation
Patterns by Pyrosequencing® Technology." Methods in Molecular
Biology, 2007, 373: 89-102.

Tusnády, G. E. et al., "BiSearch: primer-design and search tool for
PCR on bisulfite-treated genomes." Nucleic Acids Research, 2005,
33 (1): 1-6.

(Continued)

Primary Examiner — Jeanine A Goldberg

(74) *Attorney, Agent, or Firm* — Saliwanchik, Lloyd &
Eisenschen

(57)

ABSTRACT

The invention pertains to analyzing the levels of DNA
methylation at specific genetic loci to detect specific body
fluids, for example, vaginal secretions or vaginal epithelial
cells, semen or sperms, saliva or buccal epithelial cells, or
blood or blood cells. Particularly, the levels of methylation
of DNA at the genetic loci corresponding to SEQ ID NOs:
1, 6, 11, and 16 are used to detect vaginal secretions or
vaginal epithelial cells, semen or sperms, saliva or buccal
epithelial cells, and blood or blood cells, respectively. The
level of methylation at the specific loci can be determined by
high-resolution melt analysis (HRM) or sequencing of the
amplicons produced using specific primers designed to
amplify the specific loci. Kits containing the primers and
reagents for carrying out the methods disclosed herein are
also provided.

10 Claims, 2 Drawing Sheets

(2 of 2 Drawing Sheet(s) Filed in Color)

Specification includes a Sequence Listing.